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TEMPERATURE RELATED CHARACTERISTICS OF PERIPHERAL NERVES OF THE PIGEON COLUMBA LIVIA EXPOSED TO DIFFERENT THERMAL CONDITIONS

A

THESIS

Presented to the Faculty of the
University of Alaska in partial fulfillment
of the requirements for the Degree of
Master of Science

By
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December, 1978

TEMPERATURE RELATED CHARACTERISTICS OF PERIPHERAL NERVES

OF THE PIGEON COLUMBA LIVIA

EXPOSED TO DIFFERENT THERMAL CONDITIONS

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ABSTRACT

The functional capabilities of two sections of pigeon leg nerves were compared with respect to temperature in three groups of pigeons exposed to different thermal conditions. The lower leg nerves from the unfeathered portion of the leg remain functional at tissue temperatures as low as -4°C. Within each group of pigeons (winter acclimatized, summer acclimated, and southern) the fast fibers of the lower leg nerves always had significantly lower action potential extinction temperatures than those of the upper leg nerves. The conduction velocity-temperature curves show that the upper leg nerves of all three groups are similar; that the lower leg nerves of summer and winter birds are similar; and that the lower leg nerves of southern birds are significantly different from the other two groups at 34°C and at the extinction temperature. This indicates that southern birds are not as well adjusted to cold as either summer or winter birds.

ACKNOWLEDGMENTS

I would like to thank Drs. Dale Feist and Ron Smith for their helpful comments in reviewing this thesis and for being on my graduate committee, along with Dr. Pat Andresen who acted as my outside examiner. Dr. Charles Geist was also of great assistance in helping me run a computer program which gave me a better understanding of my data. I also want to thank Joe Galath for donating most of the pigeons used in this study, the animal quarters personnel for helping me care for them, and I especially want to thank Lynda Bashor, for proofreading this thesis and for moral support. Above all, my deepest thanks and appreciation goes to Dr. L. Keith Miller, my graduate advisor and committee chairman, whose invaluable assistance and advice made this thesis possible and in fact enjoyable.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Cont.)

INTRODUCTION

Birds and mammals of interior Alaska are exposed to ambient air temperatures which range from a winter low of approximately -55°C to a summer high of 36°C. This rather extreme temperature range poses unique thermoregulatory problems to resident endotherms.

The extreme cold during the winter forces both birds and mammals to be well insulated in order to avoid excess heat loss. However, these endotherms must not only avoid excess heat loss during the winter but must also have mechanisms for heat dissipation during activity or when exposed to relatively high summer temperatures. The effective insulation for heat conservation makes the problem of heat dissipation more difficult. In dealing with this problem, to at least some degree, many endotherms use their bare or lightly insulated extremities to radiate excess heat.

The importance of bare or lightly insulated extremities in thermoregulation has been investigated or referred to in a number of studies. Irving and Krog (14) found that the variability of temperature was four times greater on bare extremities than on the skin of well insulated bodies. They also recorded tissue temperatures in the more peripheral parts of these bare extremities as low as 0°C, with an ambient air temperature of -15°C or lower, while deep body temperature remained in the normal range.

Baudinette, *et al.*, (3) found that the rate of heat loss through the feet of gulls was from 37 to 52% of total body heat. They concluded that, "all of the heat produced in gliding flight and most of

the heat from flapping flight can be lost by transfer from the feet and that none need be stored". Bernstein (4) found evidence of a "rete mirabile", (countercurrent heat exchanger) under neural control, operating in the upper, feathered portion of pigeon legs. Steen and Steen (38) found that less than 10% of the metabolic heat is lost through the legs of herons and gulls at low ambient temperature but that almost all the metabolic heat production is dissipated through the legs at 35°C. They also found that the degree of heat loss changes within seconds in response to changing temperature. This latter finding would seem to disagree with that of Necker (31) who found the glabrous skin of pigeon legs to be insensitive to non-painful thermostimulation. In partial explanation of this apparent discrepency, he says that, "it cannot be excluded that this procedure (of Steen and Steen, 38) caused rapid changes in core or spinal cord temperature due to the heat exchange function of the legs". These studies and others (16, 17, 25, 27, 28, 33, and 39) well illustrate the importance of bare or lightly insulated peripheral tissue in the thermoregulation of northern birds and mammals exposed to both high and low ambient temperatures.

The need for northern endotherms to maintain the functional integrity of exposed peripheral tissues poses certain problems at low ambient temperatures. Heat must be conserved for two reasons: (1) the metabolic cost of maintaining exposed tissues at core temperature would be exorbitant, and (2) there would be the danger of icing if the snow in contact with these tissues melted (12, 14, and 38). The

greatest problem in having tissues at low ambient temperature is that tissue temperature must still be controlled, and presumably monitored, to avoid the possibility of freeze damage.

In the process of conserving heat by peripheral vasoconstriction, a temperature gradient forms along the appendages. Ederstrom and Brumleve (9) noted that in cold exposed pheasants there is a sharp drop in temperature at what they called the knee (actually the intertarsal joint or ankle), this being the point where the feathering and most of the muscle tissue end. This gradient has also been noted by Irving and Krog (14) and Irving (12) in a variety of birds and mammals, as well as by Miller and Springer in pigeons (personal communication). Peripheral tissues range from deep body temperature to as low as 0°C.

The question of immediate interest is how do these endotherms maintain neural control at temperatures approaching 0°C? Paintal (32) concluded that "conduction is blocked in all myelinated nerve fibres at about the same temperature". He used cat vagal, saphenous, and cervical sympathetic nerves, which were all exposed to similar temperature regimes, and found that the temperatures at which conduction was blocked ranged from 5° to 15°C, with a mean of 7.6°C. If his conclusion applied to all endotherms there would be no neural control of or sensory input from tissues below 5°C. However, in comparative studies on mammalian and avian peripheral nerves, it has been shown conclusively that temperature adaptation does occur in peripheral nerves of hibernators (6, 18, and 29), non-hibernators (8, 24-28, and 33), and one species of bird (7). Such temperature adaptations enable

nerves of cold exposed tissues to function at lower temperatures than those in tissues unaccustomed to cold.

Three of the studies on peripheral nerve function as related to temperature compared hibernators to non-hibernators (6) and active hibernators to inactive hibernators (18 and 24). The much more extensive work on non-hibernators has compared peripheral with deep body nerves in the beaver (27) and in four species of seals (28); caudal nerve function as related to temperature in a variety of Alaskan mammals (26); and in cold and warm climate muskrats (25). There have also been three studies on the effects of cold exposure on peripheral nerve function in laboratory rats (8, 24, and 33).

The only comparative work done on peripheral nerve adaptations to cold in birds was done by Chatfield, *et al.* (7). In that study, two segments of the superficial peroneal nerve were compared in Herring gulls previously exposed to high, low, and intermediate temperatures, and in warm adapted hens. Their results indicate differences in temperature related function in nerves of each bird between the upper (tibial) and lower (metatarsal) segments, as well as differences between each group. The cold adapted group had the lowest extinction temperatures for their metatarsal segments of the superficial peroneal nerve. The results from the hens compared very well to the results for the heat adapted gulls. Scholander, *et al.* (37), indicated that a gull normally kept in a warm room froze the web on its feet when it escaped to a cold environment; Chatfield, *et al.* (7), observed that this may be an example of a loss of cold adaptation.

There are several changes which are known to occur in cold adapted nerves. The extinction temperature of the action potential is lowered in cold exposed (peripheral) nerves to as low as 1°C in gulls (7) and, by extrapolation from the temperature at which spontaneous freezing occurred, to -9°C in seals (33). In cold adapted nerves at low temperatures, the rate of change of conduction velocity with temperature is lowered, threshold values are lower, absolute refractory periods are shorter, and the amplitude of the action potential is higher.

The present study was designed to examine possible temperature adaptations in peripheral nerves of pigeons, to correlate the relative degree of adaptation with tissue temperature data on pigeons (30) and other birds (9, 12, and 14), and to compare these results with the responses of a group of pigeons from a warmer climate which had never been exposed to freezing temperatures.

METHODS

Three groups of pigeons were compared with respect to nerve function: winter acclimatized (WA), "summer"* acclimated (SA), and southern (SO). Both the WA and the SA groups were obtained in the Fairbanks area while the SO birds were from the Los Angeles area.

The local birds were donated by Joe Galath (a building contractor in the Fairbanks area) in February of 1976 and were maintained in the WA state by keeping them in an outside flight cage located in back of the Institute of Arctic Biology (IAB). The data from the WA birds was collected in March and November 1976 and February 1977. The birds were constantly exposed to the Fairbanks winter and had been exposed for at least two months prior to testing. The ambient air temperature ranged from 3.9° to -46°C during the winter of 1975-76 and from 10.6° to -31°C during the winter of 1976-77 (National Weather Service data, Fairbanks).

The SA birds were taken from the flight cage in the fall of 1976, prior to the onset of winter (freezing temperatures), and moved to an inside cage in the animal quarters of IAB. The birds were kept indoors for at least one month before testing began.

The SO birds were obtained from the Los Angeles area in February 1977 through Karella's Korner, a poultry business in the Fairbanks area. The criterion used in ordering the birds was that they had not

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*These birds were acclimatized outdoors during the summer of 1976 and then acclimated to laboratory conditions that fall.

been exposed to frost. The annual average maximum temperature in the Los Angeles area is 20.7°C and the annual average minimum is 12.4°C, for an average of 16.6°C. The recorded low temperature for the Los Angeles area is 2.8°C (10). On arrival, in February 1977, the SO birds were immediately moved into the animal quarters, where they were allowed at least three weeks in which to acclimate to their new surroundings. The temperature range in the indoor cage, which applies to both the SA and the SO groups, was recorded with a Weksler maximum - minimum thermometer. It ranged from 4.5° to 28°C, for a median of 16°C, which is only 0.6°C lower than the average for the Los Angeles area. The SA and SO birds were kept under continuous illumination of varying intensity, with the brighter light occurring from approximately 8 am to 5 pm. In this way summer photoperiod was simulated in an attempt to maintain their summer and southern acclimatized states.

All three groups were fed and watered on the same schedule. Purina Pigeon Chow Checkers (Ralston Purina Co.) and water were always available, although the water for the WA birds was in the form of snow. All the birds used were adults and ranged in size from 350 to 600 gms, the average being 465 gms.

In collecting the data, eight complete sets of pelvic limb nerves were used from each of the three groups. Each complete set consisted of a left and right upper segment (the *N. fibularis*) and a left and right lower segment (the *N. fibularis superficialis*) (20). The *N. fibularis* segments were approximately 5 cm in length and the *N. fibularis superficialis* segments were approximately 4 cm in length.

The *N. fibularis* (peroneal) branches into the *N. fibularis superficialis* (superficial peroneal) and the *N. fibularis profundus* (deep peroneal). Since the *N. fibularis superficialis* was the most exposed and was the easiest to dissect, as well as having been used in an earlier temperature adaptation study (7), it was the nerve of choice in the present study.

To avoid any adverse effects of anesthetics, the birds were killed by a blow to the head, after which they were decapitated to get rid of as much blood as possible for easier dissection. A short piece of thread was attached to each end of the nerve segments for handling purposes, and for anchoring the nerve in the chamber. The *N. fibularis* was severed at the spine and the *N. fibularis superficialis* was severed at the base of the phalanges. The nerve was then separated into two segments by severing the nerve approximately 1 cm above the intertarsal joint. This was the point at which the exposed portion of the leg usually began (some of the pigeons were feathered below this point, but this feathering appeared to be only of a decorative nature, as the results later indicated).

During the dissection, which took from 1.5 to 3 hours, the nerve was kept moist by frequently bathing it with Hanks' balanced salt solution, purchased from Flow Laboratories. After removal, the nerve segments were immediately placed in a beaker of Hanks' solution at room temperature (ca. 22°C) and kept there until needed. In all cases, the four nerve segments (one complete set) were tested within 12 hours following the death of the pigeon. In subsequent rewarming

tests, nerve deterioration proved to be insignificant in all but the lower right segment, which was the last section to be recorded from in each set (see results).

When ready for testing, the nerve segments were placed in a specially constructed moist nerve chamber (designed by L.K. Miller) on platinum electrodes. The temperature of this chamber could be controlled to within ±0.2°C by circulating a coolant (methanol and water, 50:50) through both the lid and the base of the chamber.

Parameters examined were conduction velocity, excitability to stimuli of various durations, absolute refractory period, compound action potential characteristics, and extinction temperatures. Conduction velocities were determined to the first rise of the action potential. The average conduction distance was 3 cm for the lower segments and 4 cm for the upper segments. Excitability threshold, which was determined to be the least voltage at which an action potential was visible at an oscilloscope amplification of 1 mv per cm, was measured at five durations, 0.83, 0.19, 0.10, 0.06, and 0.01 msec. Absolute refractory periods were determined using paired stimuli of about 10 times threshold voltage at 0.2 msec duration. This voltage and duration will give the shortest refractory periods; longer duration or higher voltage would not have given significantly shorter refractory periods and would have caused problems with stimulus artifacts and possibly nerve damage (27).

The original design consisted of four groups of nerves (upper left and right, lower left and right) to be used to make comparisons

within and between the three groups of pigeons. Statistical analysis ultimately indicated a significant difference between lower left and lower right. Since this difference consistently indicated deterioration in the lower right group, and because the lower right segment was always the last segment to be recorded from in each individual, I excluded this group of nerves from the final analysis. This loss of function within such a short time (less than 10 hours) was surprising in view of the fact that excised seal peripheral nerves exhibit no measurable deterioration for 15 to 20 hours (28). Final statistical analysis of rewarming tests in the remaining three groups of nerves indicate that, at the 0.05 level of significance, only the southern lower group showed any significant deterioration after rewarming to 34°C. This deterioration was manifested only in threshold voltage (excitability).

All the above parameters were measured at three different temperatures (34°C, 25°C, and 17°C). Extinction temperatures were determined by allowing the temperature in the chamber to drop from 17°C to the temperature at which no clear action potential was visible on the oscilloscope at an amplification of 0.5 mv per cm and 1 volt stimulus magnitude. After the extinction temperatures were determined, each nerve was rewarmed to 34°C and retested to check for possible deterioration. Nerve conduction was rendered monophasic by crushing the nerve segments distal to the last recording electrode. The methods outlined above, with the exception of the graphs on action potential amplitude (Figures 2 and 3), necessarily measure only parameters of the fast fiber population of the nerves tested.

The experiments were performed using a Tektronix type 502A dual beam cathode-ray oscilloscope to visualize the action potential. The stimulator was a Grass model S4GR used in conjunction with a Grass model SIU 478A stimulus isolation unit. Temperature in the nerve chamber was controlled with a Lauda-thermostat type K2-RS, with methanol and water (50:50) as the circulating coolant. The coolant was circulated through the base and the lid of the nerve chamber but was never actually in the nerve chamber. Chamber temperature was recorded with a Leeds and Northrup Speedomax W multipoint recorder with a Flexelect B point selector. Pictures of the action potentials (Figure 1) were taken with a Tektronix oscilloscope camera, model C-12, using Polaroid type 47 black and white film.

For data analysis, the data were first run in the Honeywell computer, series 60 (level 66)/6000, using the Geist, Ullrich, and Pitz analysis of variance program, Anovtext. Using nested data, this program indicates where there will be significant differences in the mode of response of the nerve segments to temperature. Since this program uses nested data, I could only use it to get an idea of what the group response was and final statistical analysis had to be done by hand.

The final analysis was done using two fairly simple programs on the Hewlett-Packard 55 hand calculator. The first program gave mean, standard deviation, and standard error of the grouped data. The means and standard errors from this program were used in all the graphs and

are listed in Tables 1 and 3. The second program gave me Student's t values, which are listed, when significant, in Tables 1 and 2. (All Tables are located in the Appendix.)

RESULTS

The typical pattern of change in amplitude with respect to temperature is shown in Figure 1. Graphical representations of the compound action potential amplitude means are found in Figures 2 and 3. The amplitude of the lower leg nerves is greatest at 25°C; the upper leg nerves have their greatest amplitude at 34°C. The pattern of change in amplitude height with decreasing temperature is similar in the lower leg nerves of all three groups (Figure 2). In the upper leg nerves, amplitude consistently decreased with temperature. In both upper and lower leg nerves, the winter group showed the least amount of change with temperature and the southern group showed the greatest amount of change (Figure 3). The southern group also had the greatest amplitude at all temperatures (Figure 2). Another noteworthy pattern is that summer and southern birds have very similar reactions to temperature when comparing upper to lower leg nerves.

Data on extinction temperature is found in Table 1* and in Figures 4 and 5. Statistical analysis showed that there was no significant difference between the extinction temperatures of the upper leg nerves of all three groups of pigeons (Figure 4). The lower leg nerves are significantly different between summer and southern, and winter and southern, but show no significant difference between summer and winter birds (Table 1). In all cases, there was a highly significant difference between upper and lower leg nerves, indicating

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*All Tables are located in the Appendix

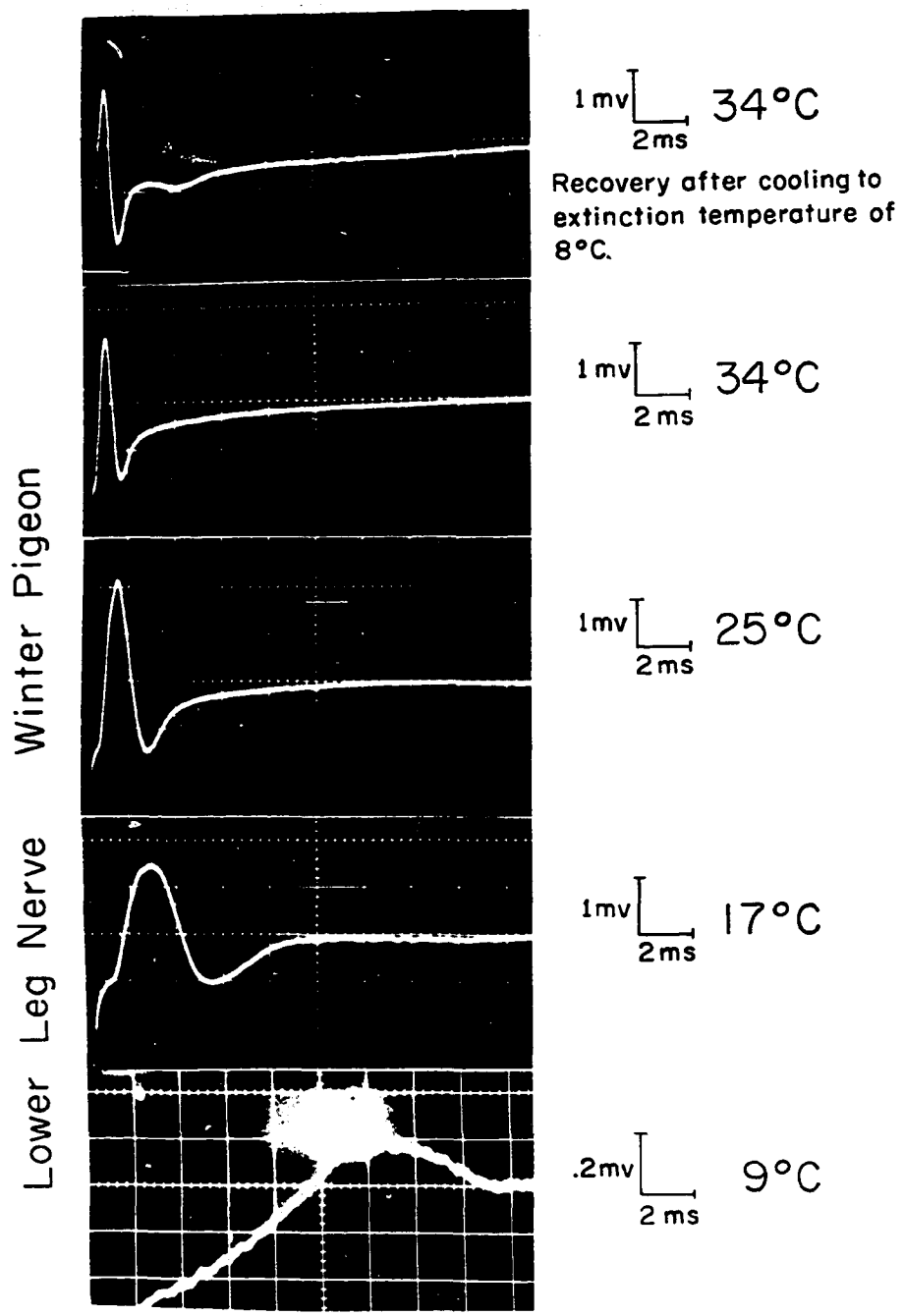

Figure 1. Typical response of action potential of a lower leg at different test temperatures in a winter acclimatized pigeon

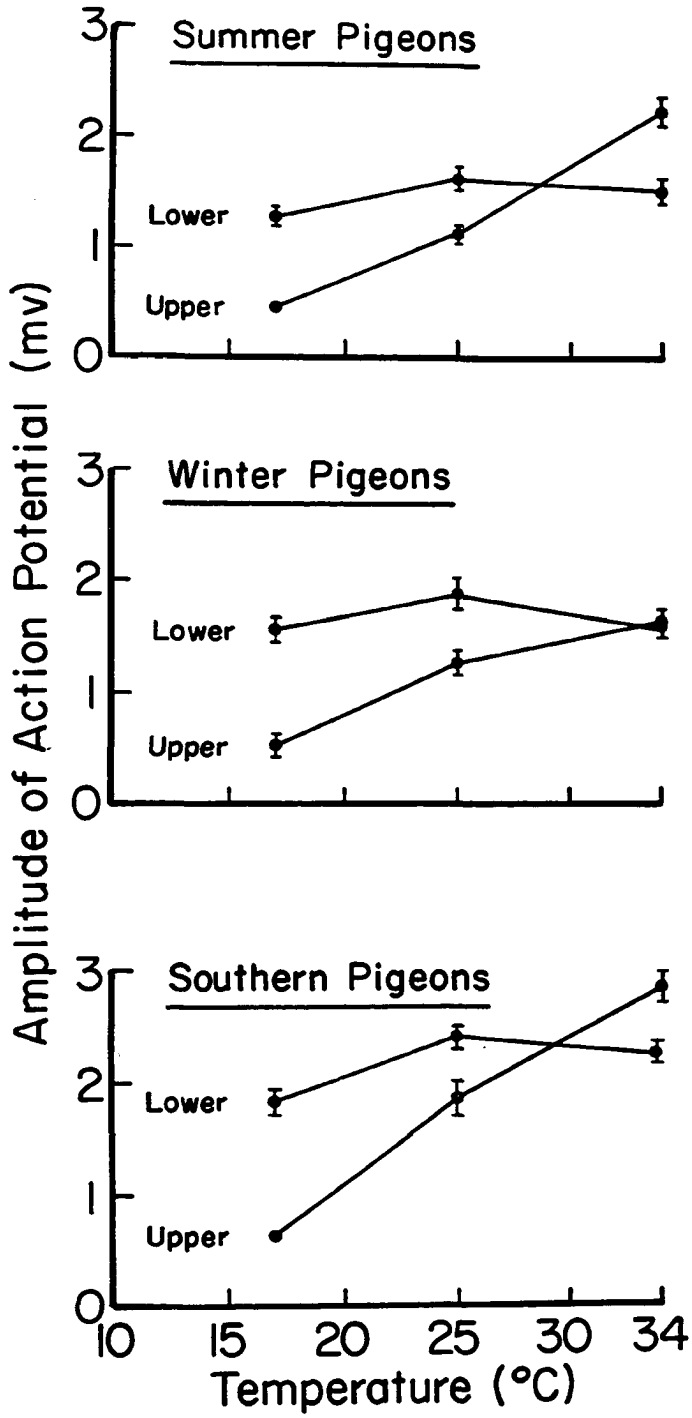

Figure 2. Summer, winter, and southern pigeon action potential amplitudes as a function of temperature in upper and lower leg nerves

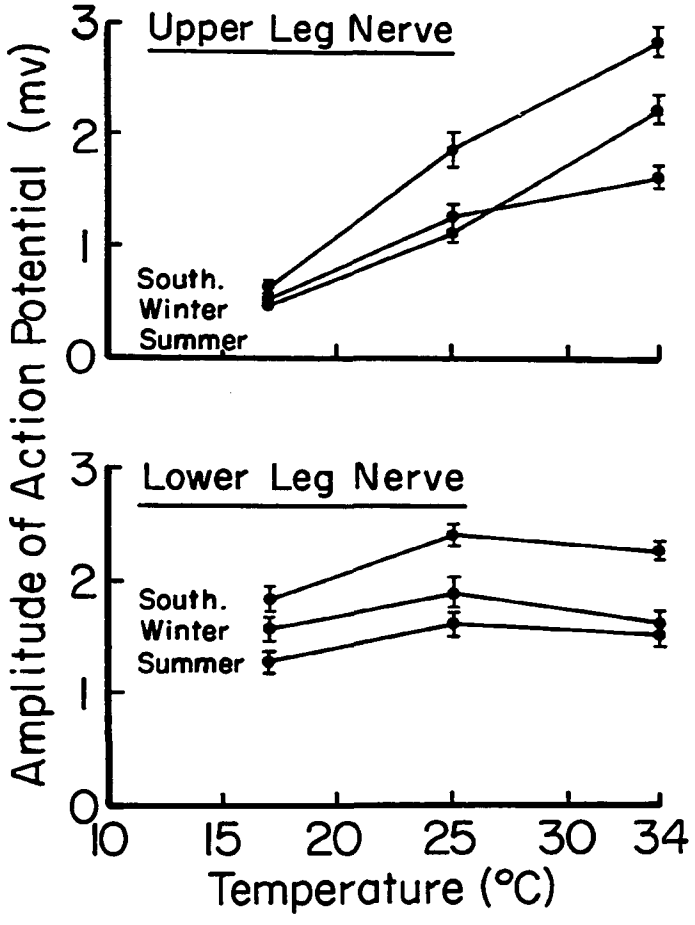

Figure 3. Action potential amplitude as a function of temperature in upper and lower leg nerves of summer, winter, and southern pigeons

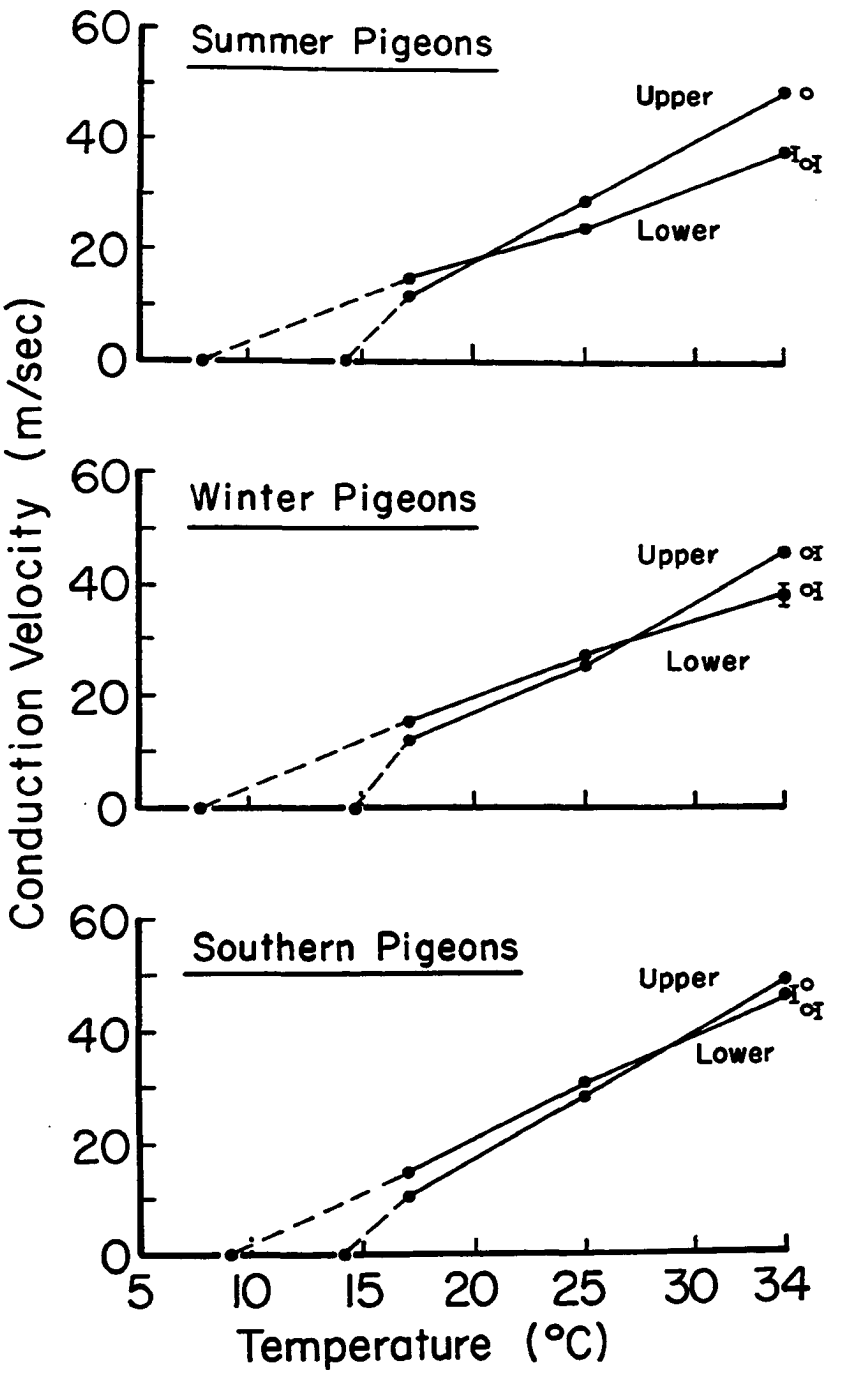

Figure 4. Summer, winter, and southern pigeon conduction velocities as a function of temperature (including extinction temperature) in upper and lower leg nerves

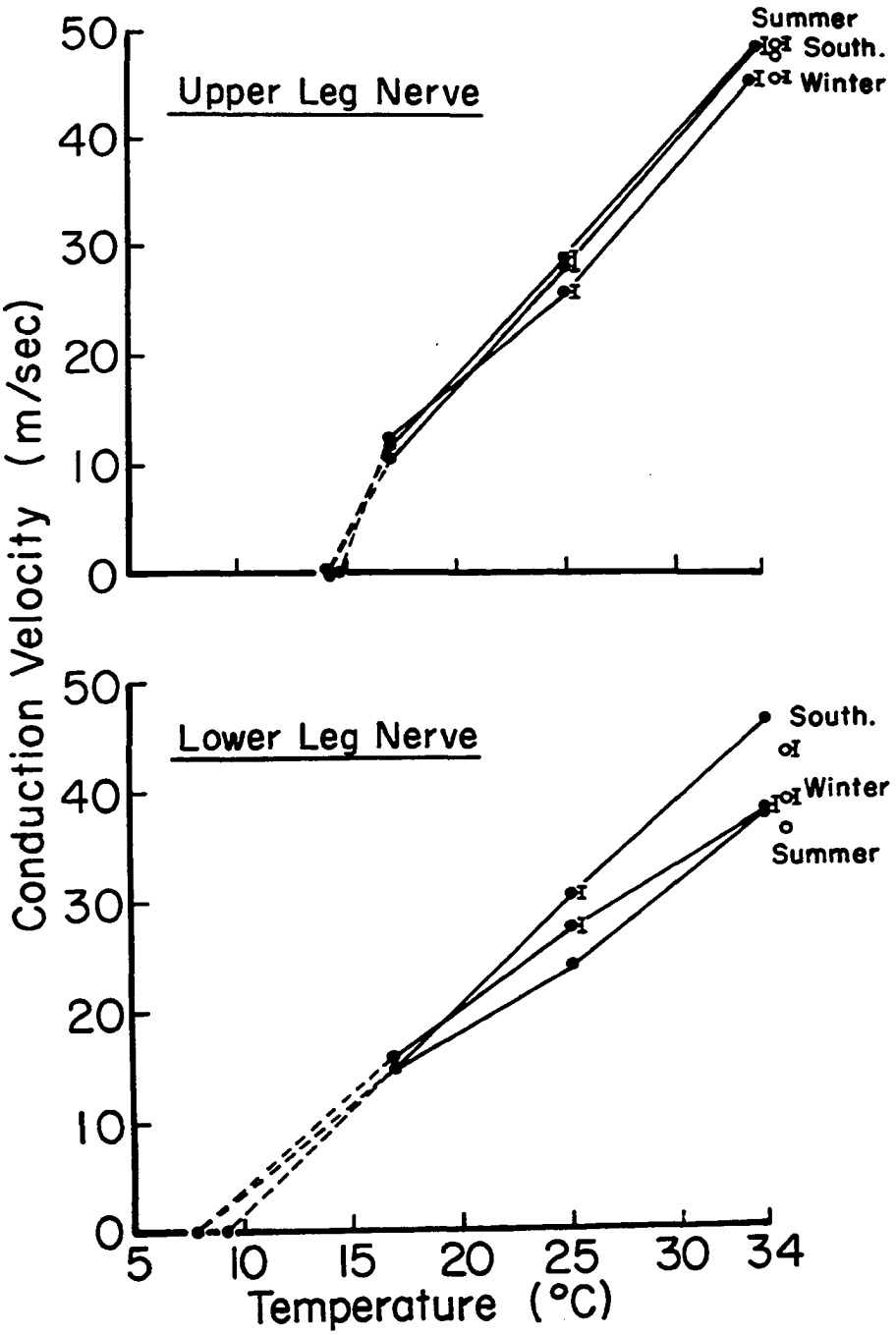

Figure 5. Conduction velocity as a function of temperature (including extinction temperature) in upper and lower leg nerves of summer, winter, and southern pigeons

alteration of function with respect to temperature within each group (Figure 5).

As shown in Figure 4, the conduction velocity of the upper leg nerves of the three groups were very similar to each other with respect to temperature and a significant difference (Table 2) was found only at 25°C between summer and winter groups. The lower leg nerves of southern birds appear to have a steeper velocity-temperature curve than either the summer or winter birds, which are statistically the same. In all three groups, when extinction temperature is included, the lower leg nerve velocity-temperature curve is more linear and appears to have a lower slope than that of the upper leg nerve.

As shown in Figure 6, there is no significant difference in excitability threshold between summer, winter, and southern upper or lower leg nerves at any temperature. When comparing upper to lower leg nerves in each of the three groups (Figure 7), the lower leg nerve is shown to be significantly better adapted to cold. There was an increasing difference in excitability between the nerve segments at decreasing temperature. Threshold at 0.01 msec duration was represented because the greatest change in threshold with respect to temperature was observed at the shortest duration.

As shown in Figure 8 in both upper and lower leg nerves the increasing length of the absolute refractory period with decreasing temperature appears to be a linear relationship in all groups. In the upper leg nerve the winter pigeons have the longest recovery period at

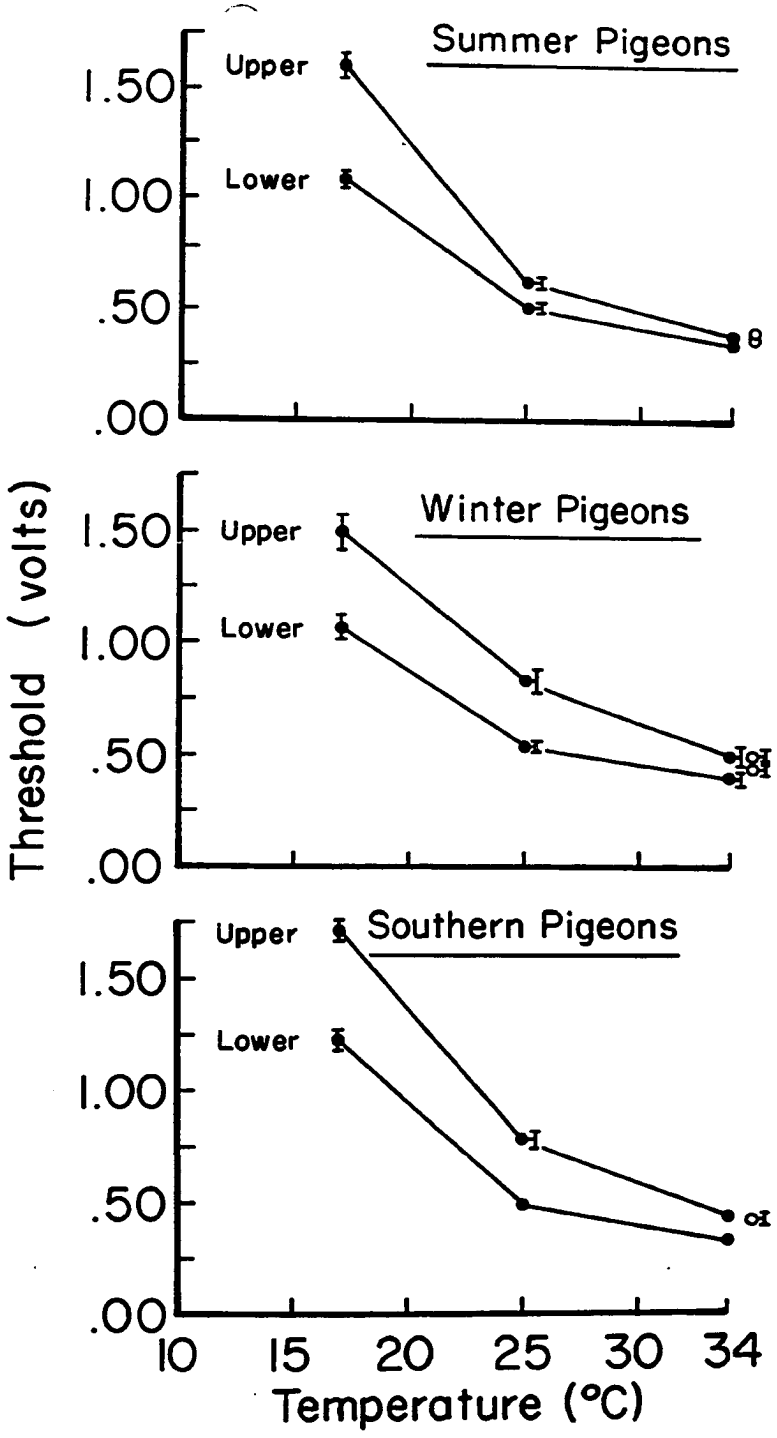

Figure 6. Summer, winter, and southern pigeon excitability thresholds as a function of temperature in upper and lower leg nerves

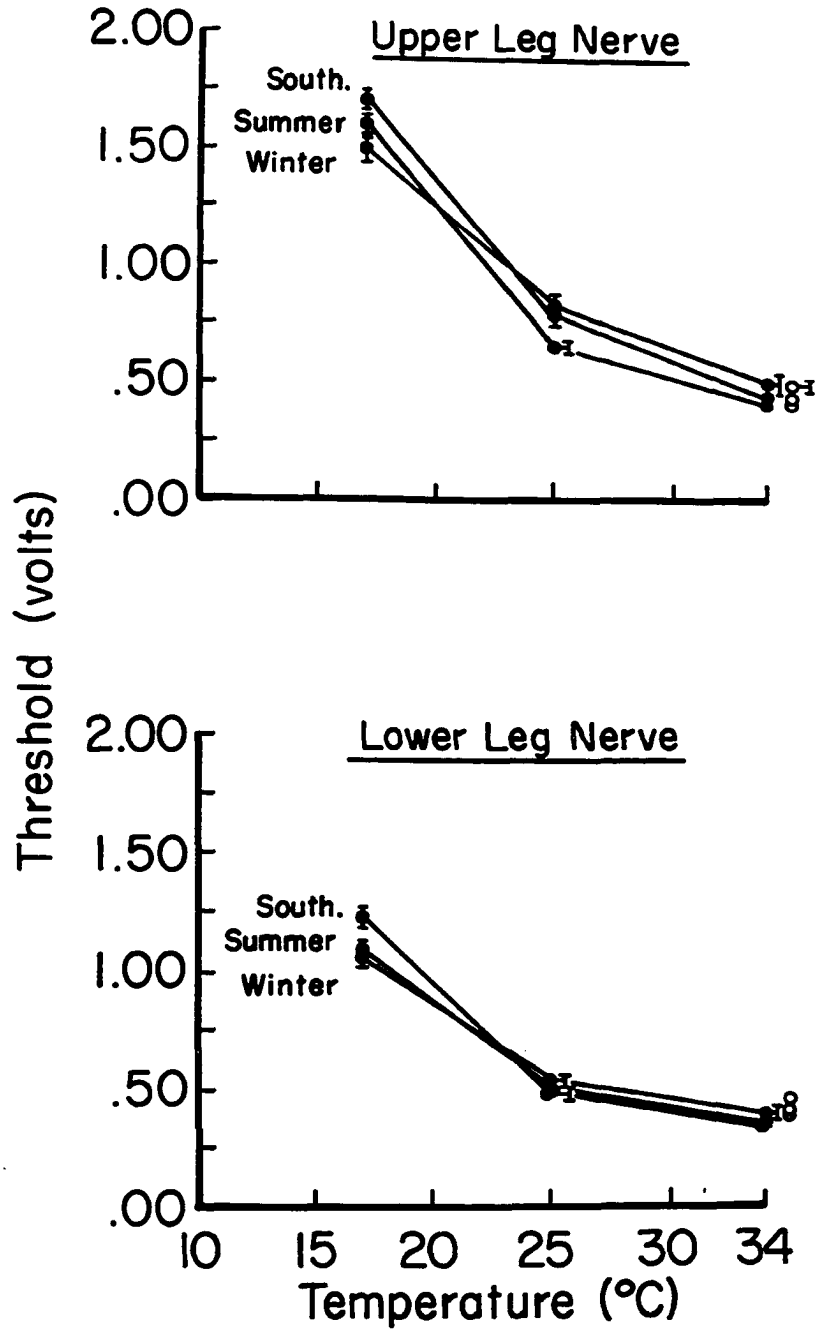

Figure 7. Excitability threshold as a function of temperature in upper and lower leg nerves of summer, winter, and southern pigeons

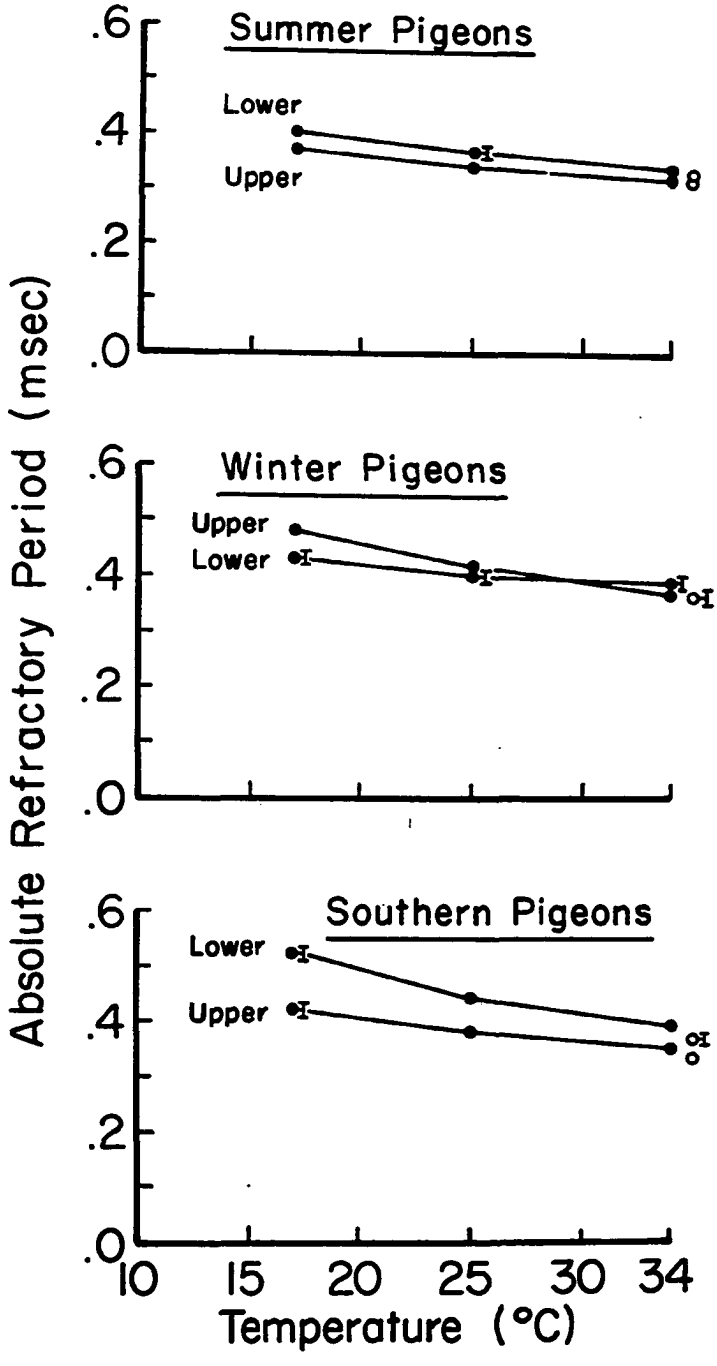

Figure 8. Summer, winter, and southern pigeon absolute refractory periods as a function of temperature in upper and lower leg nerves

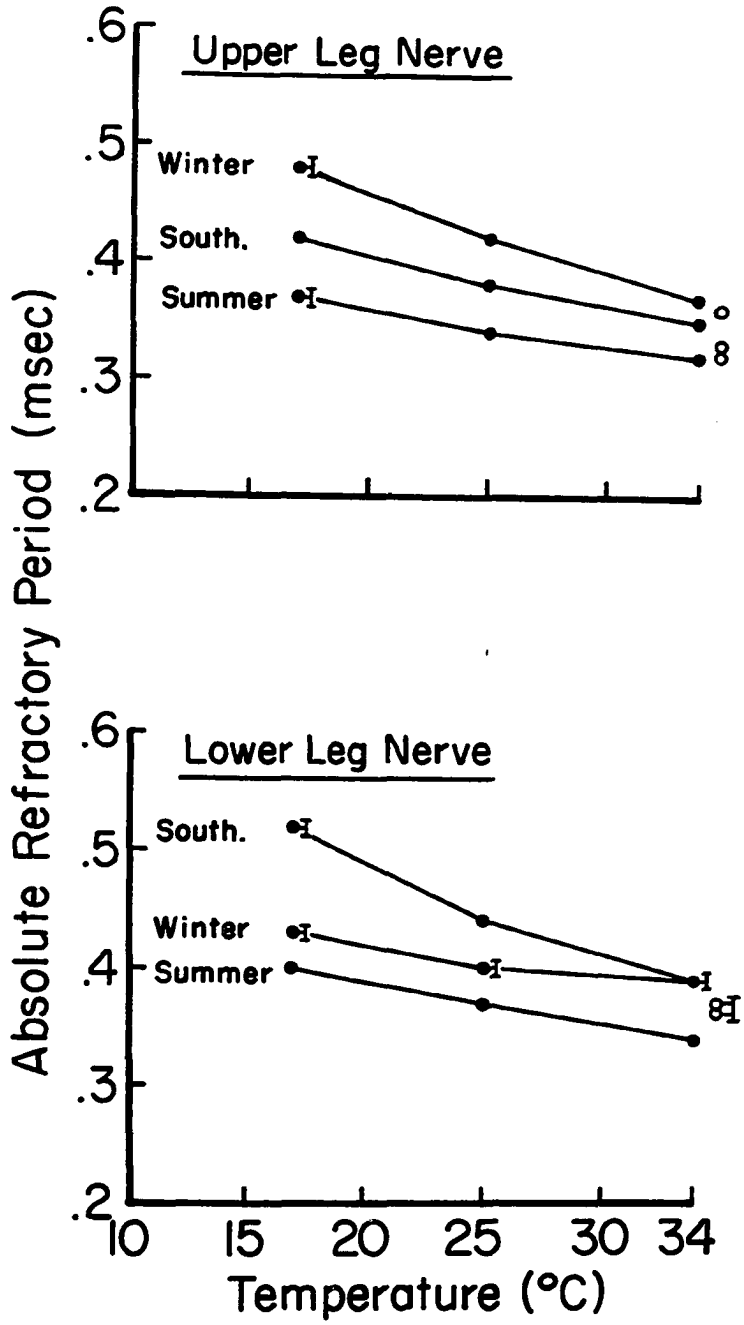

Figure 9. Absolute refractory period as a function of temperature in upper and lower leg nerves of summer, winter, and southern pigeons

all temperatures and the summer group has the shortest, with the southern group being intermediate. In the lower leg nerves absolute refractory period changes most with decreasing temperature in the southern group. This change becomes significant at 17°C (Table 2). The major point to be made is that the curves for both upper and lower pigeon leg nerves are much more linear than for mammalian nerves, and the length of the recovery period at 17°C is much shorter in pigeons than in mammals.

DISCUSSION

The results of this study definitely indicate that alterations in peripheral nerve function do occur in pigeons exposed to different temperatures.

Generally, the results of this study agree with the results of studies on mammalian peripheral nerves (8, 24-28, and 33), with the major point of difference being in the magnitude of the values. The extinction temperature of the action potential in pigeon lower leg nerves more closely resembles the extinction temperature of seal phrenic nerves (28) than any other peripheral or deep body mammalian nerve, all of which have much lower extinction temperatures. The extinction temperature for pigeon lower leg nerves is ∿8°C and the extinction temperature for seal phrenic nerve is ∿7°C. The extinction temperature for the seal lower tibial nerve is ∿-7°C (28) or 15°C lower than that for the pigeon lower leg nerves.

Conduction velocity-temperature curves are similar between pigeons and mammals except that the curves for pigeons change more rapidly with temperature. This is due to the higher extinction temperature of pigeon nerves since conduction velocities at 34°C are comparable. Excitability threshold in pigeons, as in mammals, increases with decreasing temperature, with the greatest change observed at short stimulus durations. The threshold for the pigeon upper and lower leg nerves is somewhat lower at all temperatures than that for peripheral and deep body mammalian nerves. Although it is true that threshold

measurements are highly susceptible to morphological differences and other physical variables (28), I feel that because of the number of samples ($n \bar{>} 8$) and the relatively small standard error, the differences observed here are physiologically significant.

The amplitude of the compound action potential is greatest at 25°C in pigeon lower leg nerves and is greatest at 34°C in upper leg nerves. In mammals the amplitude in both peripheral and deep body nerves is greatest at around 15°C (24, 25, 26, and 28). Chatfield, *et al.* (7) found that in gull nerves the maximum action potential amplitude occurred at ∿20°C for the metatarsal (lower) nerve and at ∿27°C for the tibial (upper) nerve. This response is about intermediate between the results for mammals and those I obtained for the pigeon.

Action potential amplitude must be analyzed with caution since whole nerve action potentials at different temperatures are subject to possible errors. Ritchie and Straub (35) in their study of rabbit nerves, concluded that "the apparently large temperature coefficient to the spike height is caused mainly by changes in the amount of dispersion and in the duration of the spike; but that there is little increase in the absolute size of the spike of the individual fibres".

The greatest difference between mammalian and pigeon nerves is in absolute refractory period. The general response is similar in that both mammalian and pigeon nerve recovery periods increase with decreasing temperature. The difference is in the rate of change of the curve and in the magnitude of the values. The recovery period-temperature curve for pigeon upper and lower leg nerves in all three

groups studied is more linear and the rate of change smaller than that for mammalian nerves. The pigeon nerve with the longest recovery period would theoretically still be able to conduct impulses at a maximum rate of 1900 impulses/sec at 17°C. (1000 msec/sec/refractory period in msec = maximum impulse frequency/sec) (27). The mammal with the shortest recorded recovery period (the muskrat) can only conduct impulses at a rate of ∿650 impulses/sec (estimated from recorded data) (20). Although this theoretical value is probably much higher than would actually be found in intact mammals or birds, the magnitude of the difference probably would not change much.

Chatfield, *et al.* (7) found a progressive difference in the degree of peripheral nerve temperature adaptation in heat adapted, warm adapted, and cold adapted gulls, as evidenced by progressively lower extinction temperatures. My results on pigeon nerves show no difference between summer and winter groups, but a significant difference between both of these groups and the southern group. Both pigeons and gulls show increasing threshold with decreasing temperature.

The fast fiber conduction velocity in the pigeon peroneal nerve is much higher (38-49 m/sec) than that in the gull (20-25 m/sec) (7) at 34°C. This difference is probably due to species differences. Lustick and Adams (22) obtained a value of 47 m/sec for a starling sciatic nerve at 34°C, and Chatfield, *et al.* (7) obtained a value of 100 m/sec for hens. Mammalian nerves have also been found to have conduction velocities in fast fibers as high as 100 m/sec at 37°C (18), so it appears that fast fiber populations in peripheral nerves

of both birds and mammals have similar ranges. A complicating factor in this study, as in other studies comparing proximal and distal segments of the same nerve, is the change in fiber composition of a given nerve as it gains or loses fibers due to branching. One can be certain that the upper peroneal contains all of the fast fibers present in the lower but it is possible that other faster fibers are also present in the upper nerve segment that are not present in the lower. The fact that the major difference in cold vs warm accustomed nerves lies in the slope or rate of change in conduction velocity with temperature, lends credence to the idea that temperature effects rather than fiber composition is the basis for the difference observed.

Most studies of temperature related changes in excitability and conduction in whole nerve trunks also suffer from a lack of information on the small fiber components. Unmyelinated fibers are especially difficult to record from, yet they usually make up a sizeable proportion of the total fiber population in any peripheral nerve. The results of this study dealing with the fast fiber population thus give information relating to the innervation of various mechanoreceptors and afferent motoneurones. Temperature related function in efferent fibers associated with temperature receptors, some nociceptors, and the autonomic system remain to be studied.

Héroux (11) found both anatomical and endocrine differences between animals exposed outdoors and those exposed to more constant laboratory conditions. There may be sufficient differences in experimental method and equipment or in nerve function in the species studied

to explain why results may vary from study to study. Therefore, it is not surprising that some differences were found between pigeon and gull nerves and between pigeon and mammalian nerves.

At the time I was preparing to do this study, Miller and Springer (30) were in the process of collecting data on tissue temperatures in pigeon legs exposed to cold. The pigeons were strapped into an apparatus which forced one leg to remain exposed. This device was then placed in a temperature chamber with temperature sensors (fine thermocouples) affixed to the leg at various points above and below the intertarsal joint, which is the joint at which the feathering usually ends. Their results show that toe temperature can fall to 0°C (and in fact to the supercooling point (∿-4°C) without permanent damage. Thus, pigeons are capable of maintaining tissue integrity via vasomotor control under rather restricted conditions. However, if not actively feeding, an unrestrained pigeon will fluff its feathers and squat on its feet when exposed to extreme cold (personal observation). Although this would indicate that unrestrained pigeons probably do not let their tissue temperature drop to this low level, a maintained toe temperature of 4°C is none-the-less reasonable in view of an extinction temperature of 7.8°C in the nerves of the metatarsal region of winter pigeons.

The fact that vasodilation accompanied by rewarming can occur in the pigeon leg at 0°C raises an important question: is such cold induced vasodilation a result of nerve function continuing in autonomic fibers or does it result from spontaneous relaxation of vasomotor

tone? Intermittent vasoconstriction, as evidenced by temperature decline, was also seen by Miller and Springer (personal communication) near 0°C in the pigeon leg.

Included in the literature on exposed peripheral tissues are several studies on the relative degree of saturation of the fatty acids in the membranes of cold exposed tissues (12, 15, 23, and 40). Saturated fatty acids have higher melting points than unsaturated fatty acids, and long chain fatty acids have higher melting points than short chain saturated fatty acids. The point here is that a long chain fatty acid with multiple unsaturations can remain fluid to as low as -50°C (21).

The studies on fatty acid composition of exposed tissues show that the more distal the tissue in a cold climate animal, the lower the degree of saturation (12, 15, 23, and 40). In other studies on fatty acid composition, it has been shown that the same loss of saturation at cold acclimation occurs in ectotherms such as frogs (2) and goldfish (5, 19, and 36). These studies demonstrate that increased unsaturation of fatty acid allows cold exposed membranes to remain functional at lower ambient temperatures.

Wilson, *et al.* (42) found that the transition points in the arrhenius plots for B-glucoside and B-galactoside transport systems are shifted by supplementing oleate and linoleate (18 carbon fatty acids with 1 and 2 points of unsaturation, respectively) into the growth media of the unsaturated fatty acid auxatroph *E. coli* K12. The transition point for both transport systems was shifted from 13° to

7°C by supplementing with linoleate. Wheeler, *et al.* (41) state that, "the electrical response of photoreceptor cell membranes appear to be a function of the position as well as a function of the total number of double bands in fatty acid supplements". Aloia, *et al.* (1), in a study of the phospholipid composition of hibernating ground squirrels, determined that, "the degree of unsaturation may not be the only factor operative in controlling membrane fluidity at reduced temperatures". They further state that, "lysoglycerophosphatides can increase the disorder of the membrane phospholipids and thus sustain a fluid environment at reduced temperatures".

The breaks in the fairly linear relationships of the velocity-temperature curves of the upper leg nerves resemble at least superficially the breaks found in the arrhenius plots of the transport systems (42). These breaks in the arrhenius plots are thought to represent a transition in the membrane lipids from a liquid-crystalline to a gel-like state (1), which entails a rapid loss of membrane function. These studies on lipid composition in cold exposed tissues as well as the fact that nerves show a peripheral adaptation to cold indicate that the nerve membrane may well undergo changes in its lipid composition on cold exposure. In both birds and mammals some insight into the mechanism of temperature adaptation in peripheral nerves may thus be gained by a close study of the lipid composition of peripheral nerve membranes.

The extinction temperatures of upper and lower leg nerves in all three groups of pigeons (Figure 5) are significantly different (Table 1).

This shows very clearly that adjustments in response to temperature do occur in the lower leg nerves of all three groups. The summer and winter groups also show a similar pattern of response (Figure 5) with the upper leg nerves having significantly higher conduction velocities than the lower leg nerves at 34°C and the lower leg nerves having significantly higher conduction velocities at 17°C (Table 2). The southern group lower leg nerves have a significantly higher conduction veolocity at 17°C but there is no difference between upper and lower leg nerves at 34°C (Table 2). Thus the lower leg nerves of the summer and winter groups adjust to temperature in a similar fashion showing a loss of function at 34°C and a gain in function at 17°C. The southern group lower leg nerves show no loss in function at 34°C but do show a gain at 17°C.

From this and other studies (6, 7, 8, 24-29, and 33), it is apparent that temperature adaptation in peripheral nerves of both birds and mammals does occur. However, the conduction velocity-temperature curves for the lower leg nerves of pigeons (Figure 4) and the statistical data (Table 2) show that there is no significant difference in the responses of the summer and winter group at any temperature including extinction temperature. This indicates that, at least in the pigeon, there is no significant change in response in lower leg nerves on a seasonal basis.

The surprising part of this study was the fact that southern birds, which had never been exposed to frost, also show a fair degree of functional capability at low temperature in their peripheral nerves.

This fact is apparent when conduction velocities and extinction temperature of lower leg nerves are compared to those of upper leg nerves (Figure 5). However, when comparing the conduction velocity-temperature curve, extrapolated to the extinction temperature, of southern pigeons to those of the other two groups (Figure 4), it becomes apparent that the lower leg nerves of southern pigeons are not as well adjusted to cold as are those of the local birds.

There are two mechanisms by which peripheral tissues may adapt to cold. One is by developing cold tolerance in exposed peripheral tissues. The other is by developing insulation on those exposed tissues, such as in ptarmigan and grouse. The local pigeons are not native to this area but were imported by man, probably within the last 20 years. Due to this relatively short time scale there is doubt as to whether the adjustments to temperature noted in the lower leg nerves of winter and summer birds constitutes a true genetic adaptation. It is not clear from the results of the present study whether the lower leg nerves of northern pigeons have increased functional capability in the cold due to the fact that they were exposed to low temperatures during their lifetime or whether a selection factor is operating. Some light could be shed on this matter by testing the temperature adaptation in a native population of birds with exposed peripheral tissues, such as the raven.

APPENDIX

Table 1. Extinction temperature and t values of upper and lower segments of Pigeon leg nerves.

	Extinction Temperature (°C)	
	Upper	Lower
SA*	14.25±0.29** (12.5-16)	7.94±0.20 (7-9)
WA	14.73±0.41 (12-17)	7.81±0.25 (7-9)
SO	14.16±0.29 (12.5-17)	9.13±0.29 (8-10)

Student t Test Values of Extinction Temperature

SA x WA	upper	-	upper x lower	SA	t = 14.15
	lower	-		WA	t = 15.50
				SO	t = 12.57
SA x SO	upper	-			
	lower	4.04			
WA x SO	upper	-			
	lower	5.32			

*SA-Summer Acclimated Pigeons
WA-Winter Acclimatized Pigeons
SO-Southern Pigeons
**Mean and Standard Error with range in parenthesis

Table 2. Results of statistical tests of nerve studies in pigeons accustomed to different environmental temperatures.

		Excitability Threshold			
		Temperature (°C)			
		17	25	34	34
SA x WA*	upper	-	-	-	-
	lower	-	-	-	-
SA x SO	upper	-	-	-	-
	lower	-	-	-	-
WA x SO	upper	-	-	-	-
	lower	-	-	-	-
upper x lower	SA	3.82**	3.76	-	-
	WA	2.52	2.10	-	-
	SO	4.30	3.91	2.44	-
		Conduction Velocity			
SA x WA	upper	-	1.78	-	-
	lower	-	-	-	-
SA x SO	upper	-	-	-	-
	lower	-	2.64	2.99	2.1
WA x SO	upper	-	-	-	-
	lower	-	-	2.34	-
upper x lower	SA	3.05	2.59	3.41	3.79
	WA	2.51	-	2.27	2.18
	SO	4.57	-	-	1.95

Table 2. (continued)

Absolute Refractory Period

		Temperature (°C) 17	25	34	34
SA x WA	upper	3.58	6.45	4.79	3.95
	lower	-	-	2.23	-
SA x SO	upper	2.40	3.22	2.16	-
	lower	5.10	2.80	3.18	-
WA x SO	upper	-	2.57	-	2.80
	lower	3.57	-	-	-
upper x lower	SA	2.01	2.26	-	-
	WA	-	-	-	-
	SO	3.57	3.44	-	-

At a 0.05 level of significance, t values must excede 1.76 or there is no significant difference (indicated by a -) between the means in question.

*SA-Summer Acclimated Pigeons
WA-Winter Acclimatized Pigeons
SO-Southern Pigeons
**t test values

Table 3. Excitability threshold, conduction velocity, and absolute refractory period of upper and lower segments of pigeon leg nerves at different test temperatures.

		Temperature (°C)			
		17	25	34	34
Excitability Threshold					
SA*	upper	1.61±0.11**	0.66±0.03	0.39±0.016	0.41±0.018
	lower	1.09±0.069	0.51±0.022	0.35±0.019	0.39±0.017
WA	upper	1.5 ±0.14	0.83±0.096	0.50±0.073	0.49±0.067
	lower	1.07±0.103	0.54±0.030	0.40±0.046	0.45±0.029
SO	upper	1.72±0.079	0.78±0.051	0.44±0.028	0.43±0.025
	lower	1.23±0.081	0.49±0.023	0.33±0.022	0.42±0.038
Conduction Velocity					
SA	upper	11.98±0.59	24.2 ±1.33	39.43±2.05	49.43±2.05
	lower	15.0 ±0.83	24.21±0.52	38.44±1.94	36.38±2.61
WA	upper	12.24±1.10	25.83±1.36	46.04±1.83	46.04±1.83
	lower	15.89±0.95	27.78±2.12	38.54±2.92	39.17±2.55
SO	upper	10.82±0.48	28.57±0.95	49.38±0.63	48.33±1.18
	lower	14.88±0.75	30.63±1.69	46.88±2.05	43.75±2.36
Absolute Refractory Period					
SA	upper	0.37±0.008	0.34±0.006	0.32±0.007	0.32±0.005
	lower	0.40±0.013	0.37±0.018	0.34±0.008	0.33±0.012
WA	upper	0.48±0.029	0.42±0.011	0.37±0.008	0.36±0.008
	lower	0.43±0.015	0.40±0.021	0.34±0.021	0.36±0.021
SO	upper	0.42±0.02	0.38±0.011	0.35±0.012	0.33±0.006
	lower	0.52±0.02	0.44±0.018	0.39±0.013	0.37±0.022

*SA-Summer Acclimated Pigeons
WA-Winter Acclimatized Pigeons
SO-Southern Pigeons
**Mean and standard error

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